A dual-stage purge-trap protocol to separate species-specific mercury from marine biota for precise isotopic analysis

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Text S1. Hg isotope measurements

The configuration and operating parameters of CVG-MC-ICP-MS are shown in Fig. S1 and Table S1, respectively.

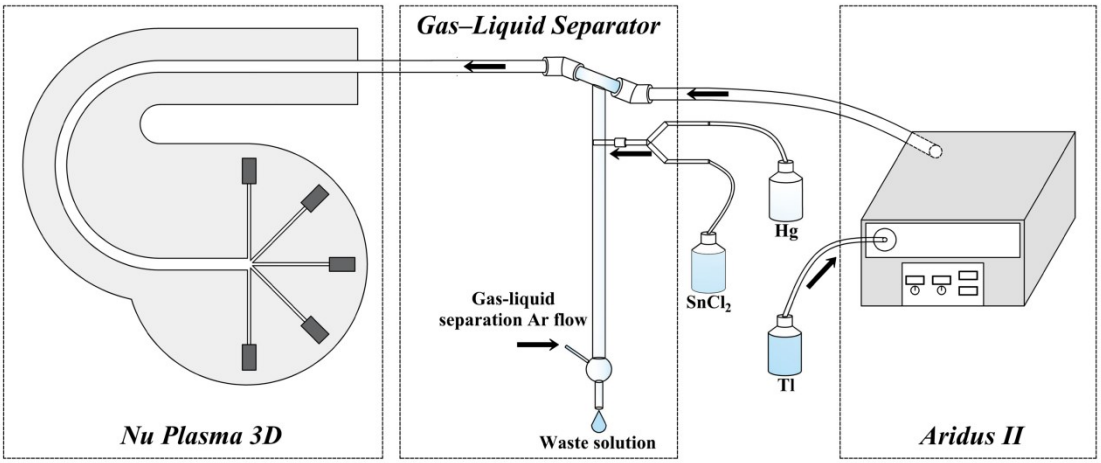

Fig. S1 Diagram of the multi-collector inductively coupled plasma mass spectrometry coupled with a customized cold vapor generation system (CVG-MC-ICP-MS)

Table S1 The operating parameters of CVG-MC-ICP-MS

Aridus II	Sweep Gas	3.2~3.8 Psi
Plasma Control	Obs In/Out	6.9~7.3 mm
	Vac Cap Position	19124~19130
	Source Temp	36 ℃
	Coolant	13 L/min
	Aux	0.95~1.3 L/min
	Neb (Press)	32~38 Psi
	Mix Gas	0.03~0.05 L/min
	Peltier Temp	14.9~15.1 ℃
	Ar Pressure	53.9~54.1 Psi
	He Pressure	0.4~0.7 Psi
Deflectors	HV 1	6001 V
	HV 2	3590~3673 V
	HV 3	3535~3565 V
	HV 4	1800 V
	HV 5	2430~2545 V
	HV 6	2075~2130 V
	Quad 1	98.2~128.9
	Quad 2	-388.6~-338
	Q1 Lin L	-12~0
	Q1 Lin H	8~12

Cup Configuration	H3	^{205}Tl
	H2	^{204}Hg
	H1	^{203}Tl
	AX	^{202}Hg
	L1	^{201}Hg
	L2	^{200}Hg
	L3	^{199}Hg
	L4	^{198}Hg

Text S2. Additional optimization of the experimental protocol

1) Optimization of $SnCl_2$ (stannous chloride) addition

In the first stage of separation and collection, we conducted experiments where different amounts of IHg (30, 60, and 90 ng) were reduced with 0.1 mL of $SnCl_2$. The recoveries were 75.8±1.2% (n=2), 33.7±0.5% (n=2), and 23.3±1.0% (n=2), respectively, suggesting that the amount of $SnCl_2$ added may be insufficient. As a result, the addition of $SnCl_2$ was increased tenfold, to 1 mL, to ensure complete reduction of IHg.

Table S2 Optimization experiments of $SnCl_2$ addition

ID	Treatments	Recovery
1	30 ng IHg+0.1 mL $SnCl_2$+8 L/h N_2, 3 h	75.8±1.2% (n=2)
2	60 ng IHg+0.1 mL $SnCl_2$+8 L/h N_2, 3 h	33.7±0.5% (n=2)
3	90 ng IHg+0.1 mL $SnCl_2$+8 L/h N_2, 3 h	23.3±1.0% (n=2)
4	90 ng IHg+1 mL $SnCl_2$+8 L/h N_2, 3 h	98.1±0.7% (n=3)

2) Optimization of purge gas flow and time

To optimize the flow rates of purging gas, we conducted experiments with flow rates of 4, 8, and 12 L/h using the IHg standard solution. The results indicated that the recoveries at 8 and 12 L/h were 94.9 ± 2.919% (n=2) and 95.1 ± 4.717% (n=3), respectively. The low recoveries at the flow rate of 4 L/h could be attributed to the tendency of Hg(0) to adhere to the internal surfaces of the apparatus and tube

connections at lower gas flow rates. Therefore, the 8 L/h nitrogen flow rate was selected. It is noted that the optimal gas flow rate may vary depending on the size and shape of the bottles.

Table S3 Optimization experiments of purge gas flow

ID	Treatments	Recovery
1	30 ng IHg+1 mL $SnCl_2$+12 L/h N_2, 3 h	95.1±4.7% (n=3)
2	30 ng IHg+1 mL $SnCl_2$+8 L/h N_2, 3 h	94.9±2.9% (n=2)
3	30 ng IHg+1 mL $SnCl_2$+4 L/h N_2, 3 h	51.6±9.1% (n=3)

Furthermore, we optimized the purging duration by setting it to 10, 30, and 60 min. The resulting recoveries were 95.3±4.3% (n=3), 94.3±5.7% (n=3), and 93.2±5.3% (n=3), respectively. Consequently, we selected the 10 min purging duration for both the first and second stages of Hg(0) trapping.

Table S4 Optimization experiments of purging gas duration

ID	Treatments	Recovery
1	100 ng IHg+1 mL $SnCl_2$+150 mL/min N_2,10 min	95.3±4.3% (n=3)
2	100 ng IHg+1 mL $SnCl_2$+150 mL/min N_2,30 min	94.3±5.8% (n=3)
3	100 ng IHg+1 mL $SnCl_2$+150 mL/min N_2,60 min	93.2±5.3% (n=3)

Text S3. Accuracy of Isotope Values for Different Types of Biological Samples

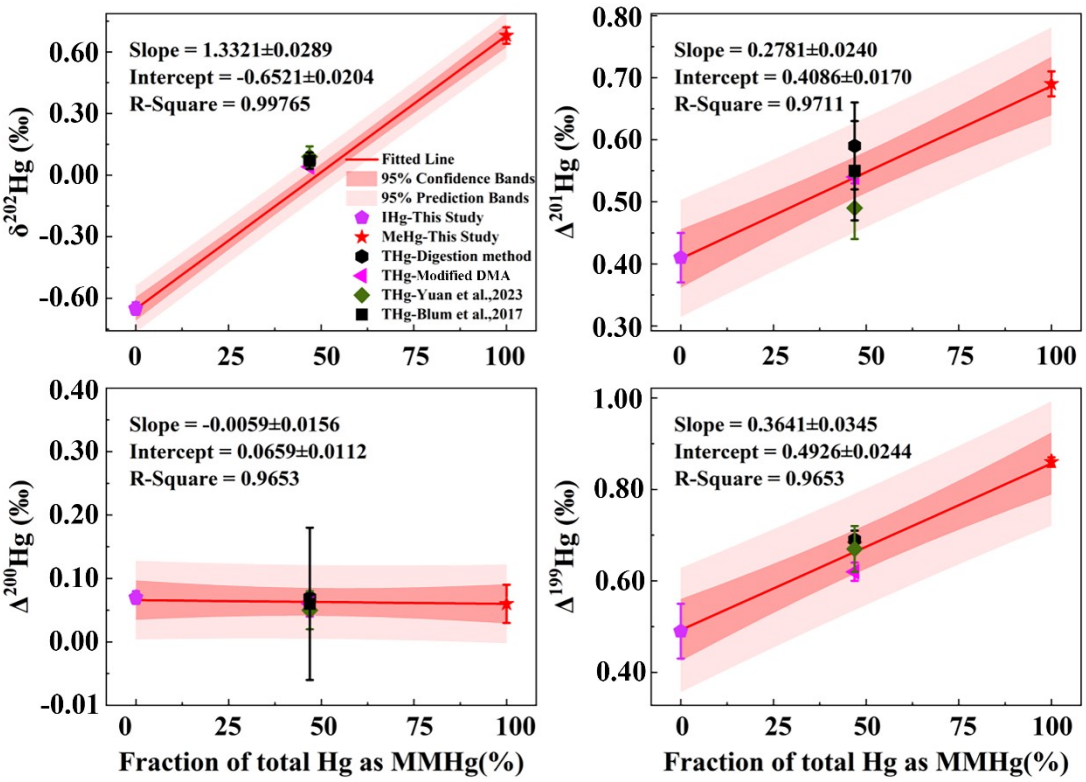

Fig. S2 Validation of the dual-stage purge-trap protocol for IHg and MeHg isotope measurement of the biological CRM TORT-3. Total Hg isotope values are based on this study and those reported previously [1, 2]. The error bars denote the 2σ analytic uncertainties. The regression lines bounded with 95% confidence bands (red, the true regression lines are likely to lie 95% of the time) and 95% prediction bands (light red, 95% of new data points are expected to fall) were fitted according to IHg (0% MeHg) and MeHg (100% MeHg) end-members measured in this study.

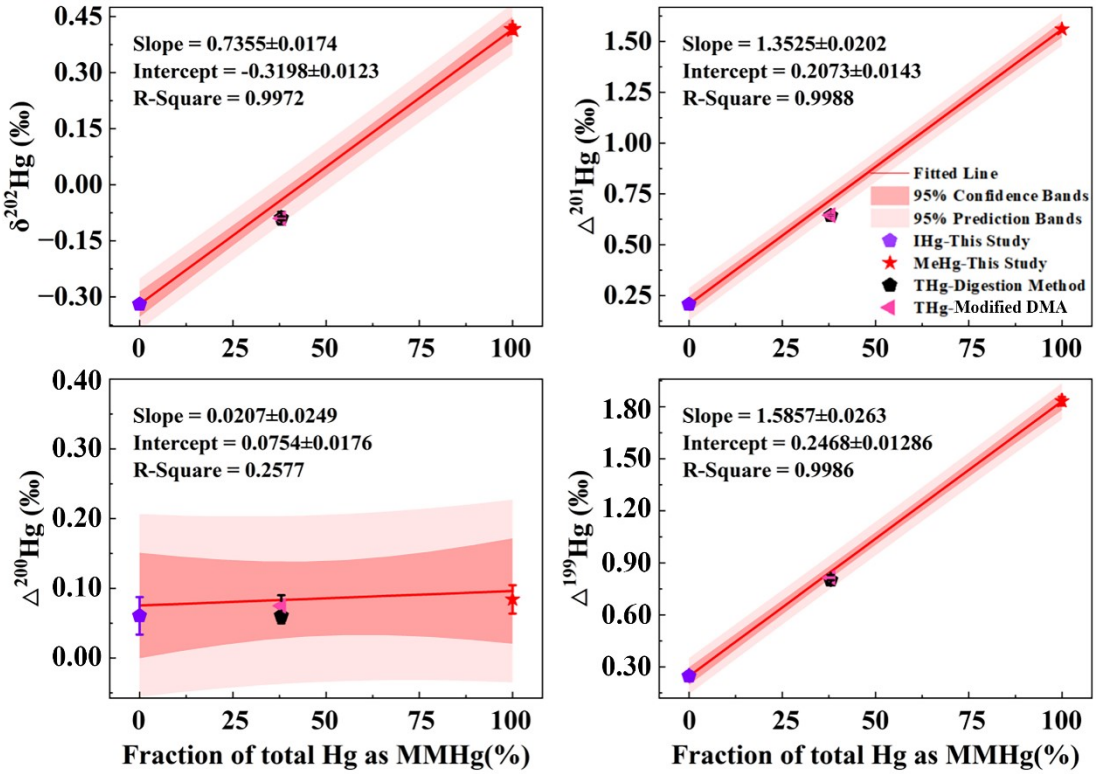

Fig. S3 Validation of the dual-stage purge-trap protocol for IHg and MeHg isotope measurement of a biological sample (mantis shrimp). Total Hg isotope values are based on this study. The error bars denote the 2σ analytic uncertainties. The regression lines bounded with 95% confidence bands (red, the true regression lines are likely to lie 95% of the time) and 95% prediction bands (light red, 95% of new data points are expected to fall) were fitted according to IHg (0% MeHg) and MeHg (100% MeHg) end-members measured in this study.

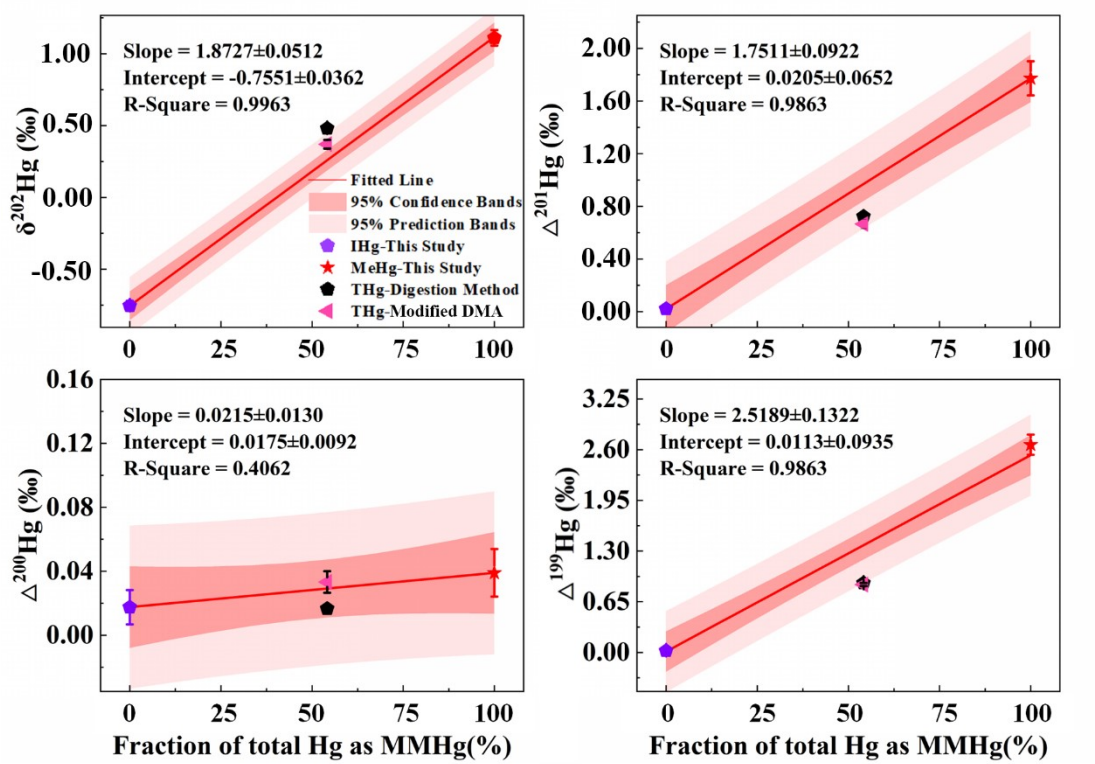

Fig. S4 Validation of the dual-stage purge-trap protocol for IHg and MeHg isotope measurement of a biological sample (portunid). Total Hg isotope values are based on this study. The error bars denote the 2σ analytic uncertainties. The regression lines bounded with 95% confidence bands (red, the true regression lines are likely to lie 95% of the time) and 95% prediction bands (light red, 95% of new data points are expected to fall) were fitted according to IHg (0% MeHg) and MeHg (100% MeHg) end-members measured in this study.

REFERENCES

1. J. Yuan, Y. Liu, S. Chen, X. Peng, Y. F. Li, S. Li, R. Zhang, W. Zheng, J. Chen, R. Sun and L. E. Heimbürger-Boavida, *Environ Sci Technol*, 2023, **57**, 6550-6562.
2. J. D. Blum and M. W. Johnson, *Reviews in Mineralogy and Geochemistry*, 2017, **82**, 733-757.